VERIFICATION OF THE TRANSLATION

I, Won-Jin HONG of 648-23, Yoksam-dong, Kangnam-ku, Seoul, Korea being fully conversant with both the Korean and the English languages, declare and swear that the attached is a true and accurate translation of Korean Patent Application No. 1999-46795 filed on October 27, 1999.

Signed by Wonjin.
Won-Jin HONG

Dated this 7th day of July, 2003

[ABSTRACT]

[Abstract]

The present invention provides sequence listings (SEQ. ID. NO. 1~24) that can be used for the diagnosis and identification of *Mycobacterium tuberculosis* and *non-tubercuolsis Mycobacteria* based on the polymorphism of the genes and provides a method for the diagnosis and identification of the *Mycobacterial* strains. According to the method of the present invention, accurate and prompt diagnosis and identification of *Mycobacterial* strains are accomplished.

[Representing Figure]

Fig. 3

[Key Word]

Mycobacteria tuberculosis, Mycobacteria

[SPECIFICATION]

[Title of the Invention]

rpoB gene fragment and a method for identifying *Mycobacterium tuberculosis* and *non-tubercuolsis Mycobacterial* strains

[Brief Description of the Drawings]

Fig. 1 is genetic map of *rpoB* gene from *Mycobacterium tuberculosis.*

Fig. 2 is a picture of PCR amplification of *rpoB* gene region (361bp) from the standard stains of *non-tubercuolsis Mycobacteria* (NTM). In this figure, each lane represents the following strains. M: DNA size marker of 50bp ladder, lane 1: M. *gordonae*, lane 2: M. *szulgai*, lane 3: M. *kansasii* type I, lane 4: M. *gallinarum*, lane 5: M. *avium*, lane 6: M. *scrofulaceum*, lane 7: M. *asiaticum*, lane 8: M. *chelonae*, lane 9: M. *moriokaese*, lane 10: M. *phlei*, lane 11: M. *pulveris*, lane 12: M. *fortuitum* type I, lane 13: M. *austroafricanum*, lane 14: M. *smegmatis*.

Fig. 3 is a picture of electrophoresis of PCR-RFLP (*Msp* I digestion) results of standard stains of NTM. In this figure, lanes M and 1-14 represent the same strains of Fig. 2, and lane 15 represents M. *marinum*.

Fig. 4 is a picture of electrophoresis of PCR-RFLP (*Hae* III digestion) results of standard stains of NTM. In this figure, lanes M and 1-14 represent the same strains of Fig. 3.

Fig. 5 is a picture of electrophoresis of PCR-RFLP (upper: *Msp* I digestion, lower: *Hae* III digestion) result of M. *kansasii* identified by Korean Institute of Tuberculosis. In this figure lane M represents size marker.

Fig. 6 is a picture of electrophoresis of PCR-RFLP (upper: *Msp* I digestion, lower: *Hae* III digestion) result of M. *chelonae* identified by Korean Institute of Tuberculosis. In this figure lane M represents size marker.

Fig. 7 is a picture of electrophoresis of PCR-RFLP (*Msp* I digestion) M. *tuberculosis*.

Fig. 8 is a picture of electrophoresis of PCR-RFLP (*Msp* I digestion) results of M.

szulgai, one of the standard stains of NTM.

Fig. 9 shows the comparison of Sequence alignments of the *rpoB* regions from *Mycobacterium tuberculosis* and *non-tubercuolsis Mycobacterial* strains.

[Detailed Description of the Invention]

[Object of the Invention]

[Technical Field of the Invention and Prior Arts]

The present invention relates to novel genes and to a method for identification and diagnosis of *Mycobacterium tuberculosis* and *non-tubercuolsis Mycobacterial* strains using the genes, more specifically, the present invention relates to rpoB gene fragments that have particularities to species of *non-tubercuolsis Mycobacterium* and a method for diagnosing and identifying *Mycobacterium tuberculosis* and *non-tubercuolsis Mycobacterial* strains using the gene fragments.

The genus of Micobacteria comprises *Mycobacterium tuberculosis*, M. *leprae* and other *Micobacteria* species called *non-tuberculosis Mycobacteria* (NTM). Since 1980s, infections of *M. avium* in the AIDS patients have increased throughout the developed countries, and a third of the people in the world are infected and 3 million persons in the world die of them per a year, which cause a great concern to treatment of the tuberculosis.

Timpe and Runyon have classified *Non-tuberculosis Mycobacteria* (NTMs) into 4 classes according to their growth tempo, shape and ability of producing pigment in 1954. That is, they are classified as a photochromogene (I) which grows slowly (generally 5-7 days) and exhibits yellow color when it grows with light; a scotochromogene (II) which exhibits yellow or orange color without light; a nonphotochromogene (III) which exhibits light yellow color or does not exhibit color when it grows with light or without light; and a rapid growers (IV) which grows rapidly compared with other mycobacterium.

However, because various pathologic syndromes regarding mycobacterium are known and new strains are identified and diagnosed, biochemical diagnoses are adopted in addition to the diagnoses using microbiological characteristics. With the microbiological and biochemical diagnoses that are presently adopted, a lot of *Non-tuberculosis Mycobacteria* can be identified and diagnosed with comparably high accuracy. However,

these conventional methods of diagnosis are useful only when the strains form colonies and take long time (8 weeks). And these methods sometimes fail precise identification because when the differences of biochemical test results are trivial, the diagnosis result can be different according to examiners who analyze the results. Therefore, only very skilled expert can obtain reliable diagnosis result, and therefore these methods are not useful for the clinical laboratories.

In addition to the method of identification based on microbiological classification and biochemical test, a method using chromatography to analyze the lipids of cell wall from a mycobacterium or using molecular biological method is used or tried for the identification of the *Non-tuberculosis Mycobacteria* in the advanced countries. The chromatography method is thought to be very efficient because its test particularity is very high; however, because this method needs a step of culturing the strains to obtain samples for the test, the procedures are usually impeded by the slow growth of the strains; and because very expensive equipments are used, its application is restricted to a reference laboratory test only in the advanced countries.

In addition, molecular biological identification method using probes prepared with such genes that show genetic variation only in a same species because maintenance of its sequence listing is high. Because this method uses PCR, this method has such advantages that can amplify target gene with a small number of stains and can shorten the time of identification. A lot of researches using molecular biological method to identify *Mycobacteria* including *Mycobacterium tuberculosis* have been actively studied.

Among these researches, as a method for identifying *Mycobacterium tuberculosis* and *non-tubercuolsis Mycobacterial* strains by using 16S rRNA gene, there is AccuProbe manufactured by Genprobe Company. This method uses rRNA:marked-DNA hybridization and has an advantage that it takes only two days to identify strains; however, because sequence listing maintenance of 16S rRNA gene is very high, sensitivity of identification to the *non-tuberculosis Mycobacteria* is low and respective kits are needed to identify different *Mycobacteria* strains(4-5). As AccuProbe products, kits for five strains of *M. tuberculosis, M. avium, M. intracellulare, M. gordonae, M. kansasii* are known, but only one strain is identified in a single experiment using these kits.

So, it is necessary to find a gene fraction having better identification character for *Mycobacterial* species than 16S rRNA and to find a method for identifying multiple strains in a single experiment, and therefore there are a lot of studies to find an improved gene fraction. As a result, rpoB gene fraction is thought to be a better gene fraction to identify *Mycobacterial* species than 16S rRNA. A precise identification of strains may be accomplished by amplifying the gene fraction with PCR then analyzing the bases of the PCR products; in addition, it would be possible to identify *Mycobacterium tuberculosis* and *non-tubercuolsis Mycobacterial* strains by using a specific restriction enzyme. Direct sequence analysis of PCR product requires expensive molecular biological equipments and highly skilled technique; therefore this method is difficult to be applied to general clinical laboratories in a general use.

Instead of analyzing the bases of the PCR products, restriction fragment length polymorphism (RFLP) analysis method, which digests the PCR products with restriction enzyme such as BestEII, HaeIII, MspI, HinfI, BsaHI or the like then compares the size and numbers of the digestion cuts to distinguish generic polymorphisms of the amplified genes so as to finally identify the *Mycobacterial* species, has been invented. This method is simpler than the method of analyzing the bases of PCR products, but has limitations that the genetic polymorphisms should be various enough to distinguish the *Mycobacterial* species in the different species and the genes should be maintained in the same species.

As the gene fractions that satisfy the above condition, *dnaJ* gene, 16S-23S rRNA spacer region 65kDa protein genetic portions are known. The PCR-RFLP method is very simple and more likely to be applied to general clinical laboratories compared with the method of analyzing the sequence listings of PCR products in viewpoint of experiment techniques. However, conventional methods require more than two restriction enzymes to obtain accurate identification; and because the difference of the sizes of gene fractions are very slight, very expensive computers and software in addition to skilled expert to analyze the gene fractions. These difficulties are originated from the facts that the polymorphisms of the target genes used in the PCR-RFLP are not sufficient to distinguish the species.

[Technical Problems that the Present Invention Is To Solve]

A gene fraction, which exists all the *Mycobacterial* species, and whose genetic conservitivity in species is high enough to identify a specific *Mycobacterium* by genetic homogeneity and whose genetic polymorphisms between species are various enough to be applied to molecular biological analysis such as PRA and PCR-DNA to distinguish the *Mycobacterial* species, is required in the identification of *Mycobacterial* species.

The present inventors have searched such gene fraction as above, and as a result, found that gene fractions corresponding to 361bp of rpoB gene are very useful to provide a rapid, simple and accurate method for identifying *non-tubercuolsis Mycobacterial* species; and the present invention accomplished based on the above find.

[Constitution and Function of the Present Invention]

The present invention provides DNA fragments including sequence SEQ. ID. NO. 1 to 4 and 6 to 24, which have genetic conservitivity of existing all *Mycobacterial* species and also have genetic polymorphisms distinguished from other species, so that can be used for identifying *non-tubercuolsis Mycobacterial* species.

In addition, the present invention provides a method for identifying and diagnosing *Mycobacterium tuberculosis* and *non-tubercuolsis Mycobacterial* strains.

The present invention provides a method for identifying *Mycobacterial* strains comprising the step of:

(1) digesting a DNA fragment which has one of the sequence Seq. ID. NO 1 to 24 with restriction enzyme to obtain DNA fragment length polymorphism pattern;

(2) isolating DNA fragment from microorganism to identify;

(3) amplifying said DNA fragment;

(4) digesting said amplified DNA fragment with the same restriction enzyme in step (1);

(5) obtaining DNA fragment length polymorphism pattern from DNA fragment in step (4); and

(6) comparing DNA fragment length polymorphism pattern from step (1) with DNA fragment length polymorphism pattern from step (5).

Alternatively, the step (1) can be performed at any step before step (6) of

comparing.

Preferably, said restriction enzymes are enzyme HaeIII or MspI. When identification is not proper by using enzyme HaeIII or MspI, enzyme Sau3A1 or BstEII may be used.

Hereinafter, the present invention described in detail together with experiments and the results thereof, but the scope of the present is restricted thereto.

MATERIALS and METHODS

1. *Mycobacterial* species

Mycobacterial species of *Mycobacterium tuberculosis* and *non-tubercuolsis Mycobacterium* are shown in Table 1.

Table 1.

Species	ATCC No.
M. abscessus	
M. asiaticum	25276
M. avium	25291
M. aichiense	27280
M. aurum	23366
M. africanum	25420
M. austroafricanum	
M. bovis	19210
M. bovis BCG	
M. chelonae	35749
M. celatum	51131
M. flavecense	
M. fortuitum	6841
M. gallinarum	
M. gilvum	43909
M. gordonae	14470
M. gastri	15754
M. haemophilum	29548
M. intracellulare	13950
M. kansasii	12678
M. malmoense	29571
M. marinum	927
M. moriokaese	
M. peregrinum	14467
M. phlei	11758

M. pulveris	
M. scrofulaceum	19981
M. szulgai	35799
M. smegmatis	
M. terrae	15755
M. tuberculosis	
M. ulcerans	19423
M. xenopi	
Nocardia brasilicros	
N. nova	
Rhodococcus	

2. DNA preparation and PCR amplification

The strains shown in Table 1 were cultured, then a loopful of bacterial colony was taken to be resuspended into 0.4ml of ddH_2O in a 1.5ml tube, and boiled for 10 minutes or sterilized for 15 minutes. Then, the resultants are centrifuged, if necessary.

The reaction composition for PCR contained 50mM of KCl, 10mM of Tris-HCl (pH 8.3), 1.5mM of $MgCl_2$, 0.001% of gelatin (w/v), each 200μM of dNTPs, 1.25U of Taq, 10pmole of primer. The samples were denatured at a denaturing temperature of 95°C for 5 min for 1 cycle; then amplified performing a cycle that includes denaturation at 94°C for 1 min, primer annealing at 58°C for 1 min and elongation at 72°C for 1 min for 35 times; and at last, the samples were incubated further at the last elongation temperature of 72°C for 10 minutes. The primer sets used in PCR amplification are as follows.

MOTTrpo-5': 5'-TCAAGGAGAAGCGCTACGA-3' (SEQ. ID. NO. 25)

MOTTrpo-3': 5'-GGATGTTGATCAGGGT CTGC-3' (SEQ. ID. NO. 26)

After the PCR, the amplification results were visualized performing 1.5% agarose gel electrophoresis using 5$\mu\ell$ of the samples and the sizes of the PCR product (361 bp) were also checked.

3. PCR-RFLP

After confirming successful PCR to obtain desired product size, 17.5$\mu\ell$ of PCR products that correspond to approximately 500ng of DNA were digested in a 20$\mu\ell$ of reaction volume using 5U (units) of *Msp* I restriction enzyme and 2$\mu\ell$ of 10X reaction

buffer solution. After more than 1 hour of incubation at 37° C, 5$\mu\ell$ of 5X DNA loading buffer was added, and then the samples were loaded into a 4% metaphor agarose gel electrophoresis to analyze the PCR product. As a DNA size marker, 100bp ladder (MBI Fermentas, Amherst, NY) was used. In addition, after performing PCR using *M. bovis* DNA as a positive control, then the *Msp* I digestion product of the PCR product was used as inner control size marker.

4. Cloning of PCR product and sequence analysis.

After performing PCR using ATCC standard strains of each NTMs, PCR products were purified by using a Geneclean (BIO101) from agarose gel and cloned into TOPO-TA cloning vector of TOTO-TA cloning kit (In vitrogen). DNA sequences of the cloned PCR products were analyzed.

RESULTS

1. PCR-RFLP analysis of *rpoB* gene using standard strains

Among the NTM standard strains deposited by Korean Institute of Tuberculosis, 33 strains of *Mycobacteria sp.* (from ATCC) and 3 strains belonging to *Nocardia sp.* and *Rhodococcus* were obtained, and genomes were separated from the DNA, then performed PCR on the *rpoB* region using MOTTrpo primer set represented by SEQ. ID. NO. 25 and 26. PCR products were visualized with agarose gel electrophoresis to find that 361 bp regions of the *rpoB* were amplified effectively, as expected (Fig. 2). The above-amplified PCR products of each strain were treated with restriction enzymes *Msp* I (Fig. 3) and *Hae* III (Fig. 4) individually, then DNA fragments were analyzed with 4% metaphor agarose gel electrophoresis to find that different size and numbers of DNA fragments were obtained according the characters of the PCR products.

2. PCR-RFLP analysis of *rpoB* gene using clinical isolates of NTM stains isolated by Korean Institute of Tuberculosis

In order to confirm whether the above PCR-RFLP results are obtained only from the standard strains of ATCC or can be obtained from clinical isolates of NTM stains that

are clinically isolated and whether the PCR-RFLP results are species-specific pattern that are conserved in the clinical isolates belonging to the same species, PCR-RFLP analyses were performed on *rpoB* gene of the strains clinically isolated. As the clinical isolates for PCR-RFLP analyses, 109 strains from 10 species, which are thought to be important in the viewpoint of clinical importance and isolation frequency, were selected from the clinical isolate strains that had been identified by strain-identification team of Korean Institute of Tuberculosis based on the microbiological and biochemical examination results. As described above, PCR was performed, PCR products were visualized, and then DNA were digested with *Msp* I and *Hae* III and finally analyzed with 4% metaphor agarose gel electrophoresis (Fig. 5 to Fig. 8).

Fig. 5 shows the result of electrophoresis obtained by PCR amplifying *rpoB* region of *M. kansasii* using DNA of the clinical strains of *M. kansasii* and MOTTrpo primer, and digesting the PCR product with *Msp* I (upper) and *Hae* III (lower), and then performing doing electrophoresis. As can be seen in Fig. 5, all the PCR-RFLP results obtained by using *rpoB* regions of every *M. kansasii* strains are the same. Therefore, it is concluded that sequence listings of *rpoB* regions of every *M. kansasii* strains are conserved and it is possible to identify *M. kansasii* by using the sequence listing of *rpoB* region of the *M. kansasii*.

Fig. 6 shows the examination result obtained by using the clinical strains of *M. chelonae*, which are similar with that of *M. kansasii*. That is, all the PCR-RFLP results obtained by using *rpoB* regions of every *M. chelonae* strains are the same; and therefore, it is concluded that sequence listings of *rpoB* regions of every *M. chelonae* strains are well conserved, which suggests that the sequence listing of *rpoB* region of the *M. kansasii* can be applied to identify *M. chelonae*.

On the other hand, because it is difficult to separate 2 subspecies of *M. chelonae*, that is *M. chelonae chelonae* and *M. chelonae absessus*, with conventional biochemical method, they are identified as a *M. chelonae* complex combining the two subspecies together; however with the result of PCR-RFLP method using *rpoB*, PCR-RFLP patterns of *M. chelonae chelonae* in lane 4 and 11 and *M. chelonae absessus* in the other lane have slight difference, and therefore, the sequence listings of *rpoB* genes of the two subspecies

were thought to be different. As a result of analyzing the sequence listings of *rpoB* genes of the two subspecies, it was found that the two subspecies could be distinguished by using restriction enzyme BstEII.

With the above two experiments, it was found that most of the differences between species were detected by the treatment of restriction enzyme *Msp* I, and that the profiles of DNA fragments treated with *Msp* I were similar in the same species, and so only *Msp* I was applied as restriction enzyme in the next experiment using clinical strains.

Fig. 7 and 8 show several examples of PCR-RFLP analysis results of *rpoB* gene regions of clinical strains of *Mycobacteria sp.*, and because restriction enzyme profiles of *Msp* I are similar in the strains of *M. tuberculosis* (Fig. 7) and *M. szulgai* (Fig. 8), which can be applied to identify strains. In addition, other experiments were performed with various NTM clinical strains such as *M. gordonae, M. avium, M. intracellulare, M. fortuitum, M. malmoense, M. scrofulaceum, M. terrae, M. bovis*, or the like (not described); and as the result, it was found that the PCR-RFLP analysis of *rpoB* gene regions of clinical strains of NTM can be used for identifying strains.

3. Sequence listing analysis of *rpoB* gene fragments of NTM standard strains having clinical importance.

Among the *non-tubercuolsis Mycobacterium sp.* connected with disease, *M. avium-intracellulare complex, M. kansasii, M. marinum, M. fortuitum, M. chelonae, M. abscessus* or the like are observed frequently, and *M. malmoense, M. asiaticum, M. xenopi, M. simiae, M. scroflaceum, M. nonchromogenicum, M. smegmatis, M. peregrinum, M. szulgai, M. haemophilum, M. ulcerans* or the like are rarely observed; in addition, *M. terrae, M. gordonae* or the like are regarded as non-pathogenic *non-tubercuolsis Mycobacterium sp.* but sometimes observed.

Among the above species, *rpoB* gene regions of 20 species (24 strains including *M. tubercuolsi*: 4 strains of *M. gordonae*) were amplified with PCR and their sequence listings were analyzed. The base lengths of rpoB genes of the strains analyzed by the present experiment were 361 bp, which is the size of PCR product, and the sequence listings of newly analyzed 235 bp from rpoB gene fragment except already known 126 bp on the C-

terminal are described in SEQ. ID. NO. 1 to 24.

4. Algorithm of NTM identification based on the results of PCR-RFLP and sequence listing analysis.

Based on the data of PCR-RFLP profile and sequence listing of PCR products of NTM standard strains and clinical strains using restriction enzyme analysis, an algorithm for identifying NTM strains was constructed (Fig. 9).

As seen in Fig. 9, gene regions that having excellent conservative and that having polymorphism are separated well. Therefore, genes from the region having exellent polymorphism can be use as signature gene to identify NTM strains.

[Effect of the Invention]

The gene and PCR-RFLP method of the present invention have a lot of advantages compared with those of conventional methods. In addition to the advantage that it can save time and efforts of the examiner due to its precise and quick identification compared with conventional molecular or biochemical methods, it can identify the strains accurately. Conventionally, it took much time about several months to obtain the results or some strains were not identified with the conventional biochemical method, but these strains can be identified accurately with the present PCR-RFLP method.

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[CLAIMS]

[Claim 1]

A DNA fragment which has one of the sequence Seq. ID. NO 1 to 4 or 6 to 24

[Claim 2]

A method for identifying *Mycobacterium* strain comprising the step of:

(1) digesting a DNA fragment which has one of the sequence Seq. ID. NO 1 to 24 with restriction enzyme to obtain DNA fragment length polymorphism pattern ;

(2) isolating DNA fragment from microorganism to identify;

(3) amplifying said DNA fragment;

(4) digesting said amplified DNA fragment with the same restriction enzyme in step (1) ;

(5) obtaining DNA fragment length polymorphism pattern from DNA fragment in step (4) ; and

(6) comparing DNA fragment length polymorphism pattern from step (1) with DNA fragment length polymorphism pattern from step (5).

[Claim 3]

A method of claim 2, wherein said DNA fragment length polymorphism from step (1) and step (5) are characterized as obtaining by electrophoresis.

[Claim 4]

A method of claim 3, wherein said restriction enzyme is characterized as *HaeIII, MspI, Sau3A1* or *BstEII.*

[Claim 5]

A method of one of the claims 2 to 4, wherein said Mycobacteria strain is characterized as *M. tuberculosis, M. avium, M. abscessus, M. flavescence, M. africanum, M.bovis, M.chelonae, M. celatum, M. fortuitum, M.gordonae, M.gastri, M. haemophilum,*

M.intraecllulare, M. kansasii, M. malmoense, M. marinum, M. szulgai, M. terrae, M. scrofulaceum, M. ulcerans or *M. xenopii.*

[FIGURES]

[Fig. 1]

[Fig. 2]

[Fig. 3]

[Fig. 4]

[Fig. 5]

[Fig. 6]

[Fig. 7]

[Fig. 8]

[SEQUENCE LISTINGS]

<110> LEE, Hyeyoung

<120> Genes specific for species of Mycobacteria and a method for diagnosis, identification and characterization of M. tuberculosis and other Mycobacteria

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ccatcgagta cctggtgcgg ctgcacgagg gtcagtcggc gatgacggtt cccggcggcg 180
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<213> Mycobacterium gordonae III

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ccatcgagta cctggtccgt ctgcacgagg gtcagcacac gatgaccgtt ccgggcggca 180
ccgaggttcc ggtggagacc gacgacat 208

<210> 4
<211> 207
<212> DNA
<213> Mycobacterium gordonae IV

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ccatcgagta cctggtccgc ctccacgagg gtcagcacac gatgacgttc cgggcgggac 180
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<210> 5
<211> 208
<212> DNA
<213> Mycobacterium tuberculosis

<400> 5
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ccatcgaata tctggtccgc ttgcacgagg gtcagaccac gatgaccgtt ccgggcggcg 180

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<210> 6

<211> 208

<212> DNA

<213> Mycobacterium terrae

<400> 6

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ccatcgaata tctggtccgc ttgcacgagg gtcagaccac gatgaccgtt ccgggcggcg 180

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<210> 7

<211> 214

<212> DNA

<213> Mycobacterium chelonae

<400> 7

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tcgccaccat cgggtacctg gtgcgcctgc acgagggcca gaccacgatg accgcccccg 180

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<210> 8

<211> 208

<212> DNA

<213> Mycobacterium kansasii

<400> 8

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ccatcgagta tctggtccgc ctgcacgagg gccaggccac gatgaccgtg ccgggcgggg 180

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<211> 223

<212> DNA

<213> Mycobacterium scrofulaceum

<400> 9

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ccatcgaata cctggtccgg ctgcaccacg cccgtacgga tggccagccc gccgtcatga 180

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<210> 10

<211> 208

<212> DNA

<213> Mycobacterium ulcerans

<400> 10

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ccatcgaata cctggtccgc ttgcacgagg gccagaccgc gatgaccgct ccgggcggtg 180

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<210> 11

<211> 208

<212> DNA

<213> Mycobacterium marinum

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<210> 12

<211> 207

<212> DNA

<213> Mycobacterium szulgai

<400> 12

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<210> 13

<211> 223

<212> DNA

<213> Mycobacterium gastri

<400> 13

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<212> DNA
<213> Mycobacterium malmoense

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<210> 15
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<212> DNA
<213> Mycobacterium avium

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<210> 16
<211> 208
<212> DNA
<213> Mycobacterium bovis

<400> 16

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<210> 17

<211> 208

<212> DNA

<213> Mycobacterium celatum

<400> 17

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<210> 18

<211> 211

<212> DNA

<213> Mycobacterium flavescens

<400> 18

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ccaccatcga gtacctggtg cggctgcatc agggcgacaa gacgatgacc gtcccgggtg 180

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<210> 19

<211> 208

<212> DNA

<213> Mycobacterium fortuitum

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<211> 205

<212> DNA

<213> Mycobacterium intracellulare

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<210> 21

<211> 214

<212> DNA

<213> Mycobacterium abscessus

<400> 21

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<210> 22
<211> 208
<212> DNA
<213> Mycobacterium africanum

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<210> 23
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<212> DNA
<213> Mycobacterium haemophilum

<400> 23
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<210> 24
<211> 208
<212> DNA
<213> Mycobacterium xenopi

<400> 24

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<210> 25

<211> 19

<212> DNA

<213> Artificial Sequence

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<400> 25

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<210> 26

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR amplification primer for amplifying the gene of strains of Mycobacterium

<400> 26

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